was reduced to 22% for grade 2-4 acute GVHD (only 4%: grade 3-4) with no increase in relapse (17% at 1-year).
Conclusions: These data demonstrate translation of our experimental observations into a novel proof-of-concept phase I/II clinical trial of vorinostat in reducing the incidence of GVHD.

3

Inhibition of c-Rel Activity Prevents Graft-Versus-Host Disease without Compromising Tumor Immunity

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Using methods to inhibit the NFkB family member c-Rel, a transcription factor that upon antigen receptor triggering regulates lymphocyte survival and proliferation, we developed a novel strategy to diminish alloactivation of T cells while preserving GVT activity.

We investigated the role of c-Rel during GVHD in MHC mismatched as well as MHC matched, minor antigen mismatched murine HSCT models. c-Rel$^{-/-}$ T cells caused significantly less GVHD than normal T cells, as determined by survival ($p<0.001$), clinical GVHD score ($p<0.01$), and histopathology of GVHD target organs. On day 3 post transplant, proliferation and activation of c-Rel$^{-/-}$ T cells was impaired ($p<0.001$ CFSE$^{low/high}$ ratio; $p<0.01$ CD25^{+} donor cells) and pSTAT5 expression was decreased ($p<0.05$), resulting in less expansion of donor derived effector T cells by day 7 after transplant ($p<0.001$). Unexpectedly, serum levels of IL-2 were increased on day 7, likely secondary to decreased pSTAT5-mediated negative feed back on IL-2 secretion. In addition, IL-21, a target gene of c-Rel and negative regulator of IL-2, was decreased ($p<0.05$) in recipients of c-Rel$^{-/-}$ T cells during early GVHD. By day 14 post BMT we observed increased numbers of donor derived Tregs with increased CD25 expression in recipients of c-Rel$^{-/-}$ T cells ($p<0.05$), suggesting that the increased IL-2 levels primarily benefited the expansion of Tregs. Consistent with this hypothesis, in vivo depletion of donor Tregs in recipients of c-Rel inhibited Foxp3DTR transgenic T cells exacerbated GVHD.

We next evaluated if c-Rel deficient T cells were able to mediate antitumor activity. We challenged allogeneic HSCT recipients with either a liquid tumor (A20) or a solid tumor (RENCA) and found that GVT activity in c-Rel$^{-/-}$ T cells recipients was intact in the absence of GVHD, resulting in significantly improved survival compared to recipients of wildtype T cells ($p<0.001$ A20; $p<0.01$ RENCA). Using syngeneic GVT models with EL4 as well as B16 we could demonstrate strong anti-tumor activity of c-Rel deficient polyclonal T cells targeting EL4 as well as melanoma antigen-specific pmel-1 transgenic T cells in the absence of T cell alloactivation, reinforcing the notion of separation of GVHD from GVT activity through inhibition of c-Rel signaling.

The potential for clinical translation of our approach is highlighted by a series of experiments testing the efficacy of a recently developed Pyrimidinetrione-based small molecule c-Rel inhibitor compound. Using T cells that were pre-incubated with this c-Rel inhibitor, we reproduced our above described effects on GVHD and antitumor activity (Figure 1).

Taken together, our findings identify c-Rel as a promising target for the development of a clinical strategy to prevent GVHD while preserving GVT activity, and to possibly even enable adoptive T cell therapy across MHC barriers that would be incompatible with a conventional transplant approach.

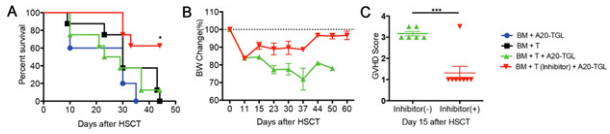

Figure 1. Pre-incubation of donor T cells with a c-Rel antagonist prevents GVHD while preserving GVT activity.
(A-C) Lethally irradiated (8.5Gy) BALB/c recipients received 5×10^6 WT B6 TCD-BM cells with 0.5×10^6 WT B6 CD5$^+$ T cells after 24 hours of pre-incubation with a c-Rel antagonist. Control mice received 5×10^6 WT B6 TCD-BM cells with 0.5×10^6 WT B6 CD5$^+$ T cells that were pre-incubated with empty vehicle control solution. On day 0, HSCT recipients were challenged with 0.25×10^6 luciferase-expressing A20-TGL tumor cells. Data are representative of at least two independent experiments; n = 5-8. Error bars indicate SEM. $^*P<0.05$; $^{***}P<0.001$. (A) Survival curve. (B) Body weight changes. (C) Clinical GVHD scores on day + 15.

CIBMTR BEST ABSTRACT AWARDS FOR CLINICAL RESEARCH

4

Improved Survival with Intravenous Busulfan (IV BU) Compared to Total Body Irradiation (TBI)-Based Myeloablative Conditioning Regimens: A CIBMTR Prospective Study

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Historical data are conflicting as to whether superior outcomes occur with cyclophosphamide (CY) + TBI compared to oral busulfan (BU)-based myeloablative conditioning regimens. IV BU with or without pharmacokinetic monitoring is better tolerated than oral BU. To test whether myeloablative IV BU-based conditioning regimens result in similar outcomes to traditional TBI-based conditioning, we conducted a prospective multi-center cohort study comparing these 2 approaches in patients with myeloid malignancies (AML, MDS, CML) undergoing matched related or unrelated donor blood or marrow transplants. All patients received myeloablative conditioning intensity (IV BU (>9mg/kg) plus Cy (≥60 mg/kg) or fludarabine (≥80 mg/m^2); TBI (≥500cGy in a single fraction or ≥800 cGy fractionated) plus Cy (≥60 mg/kg) or etoposide (≥30 mg/kg)) and calcineurin-inhibitor based

graft-versus host disease (GVHD) prophylaxis. The primary objective was to test the non-inferiority of overall survival after IV BU compared to TBI. From March 2009 to February 2011, 1,483 eligible patients were enrolled (IV BU, n=1025, TBI, n=458) from 120 transplant centers. The 2 cohorts were similar with respect to median age (45 years), gender (50% female), race (88% Caucasian), performance status (68% ≥90), and HCT-CI. Most patients had acute myeloid leukemia (68% BU, 78% TBI). Disease status was early (51%), intermediate (18%) and advanced (31%) and were balanced in both cohorts. Grafts were primarily PB (77%) from matched sibling (40%) or well-matched unrelated donors (48%), balanced in both cohorts. IV BU regimens were CY (59%) or Flu (41%) based. 2-yr probabilities of overall survival (95% CI), were 56% (53-60%) and 48% (43-54%) for IV BU and TBI, respectively (p=0.02). Corresponding probabilities of progression-free survival (PFS) were 49% (45-52%) and 44% (40-49%), (p=0.17). The incidence of hepatic veno-occlusive disease (VOD)/sinusoidal obstruction syndrome (SOS) was 5% for BU and 1% for TBI; ($p < 0.001$). Outcomes were the same for BU+CY and BU+Flu regimens. There were no differences in engraftment of neutrophils or platelets, disease relapse, grade 2-4 acute or chronic GVHD. Multivariate Analysis (MVA) (adjusting for HCT-CI, disease and status, donor type, age, PS, and race) of the main outcomes is summarized in Table. Compared to TBI, IV BU was associated with superior survival and no increased risk of relapse or TRM. These results support the use of myeloablative BU- over TBI-based conditioning regimens for treatment of AML, MDS, and CML.

Table
Cox Proportional Hazards MVA for IV BU vs. TBI

Outcome	N	IV BU vs. TBI HR (95% CI)	P-value
OM	1439	0.82 (0.68-0.98)	0.026
TRM	1434	0.81 (0.61-1.08)	0.15
Rel	1434	0.93 (0.76-1.12)	0.43
TF	1434	0.90 (0.77-1.06)	0.19

HR indicats hazard ratio; N, number of subjects; OM, overall mortality; Rel, relapse; TF, treatment failure, relapse or death; TRM, treatment-related mortality.

5

Safety and Clinical Efficacy of Rapidly-Generated Trivirus-Directed T Cells After Allogeneic Hematopoietic Stem Cell Transplant

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We have previously demonstrated that small numbers of *ex vivo*-expanded, trivirus-specific T cells targeting EBV, CMV, and Adv are safe, effective and protective *in vivo*. However, broader implementation is limited by the need for infectious virus/vector, and prolonged (8-12wks) and complex manufacture, while antigenic competition limits extension to additional viruses. We now evaluate whether T-cell lines manufactured using methods that exclude viral components and utilize simplified manufacturing technology can be clinically effective. With NHLBI-PACT support, 29 clinical-grade rCTL lines have been generated. From an initial $15x10^6$ PBMCs, we prepared a median of $214\pm88x10^6$ T-cells (range 100-$420x10^6$) over 9-11 days using DCs nucleofected with DNA plasmids encoding immunogenic EBV (LMP2, EBNA1 and BZLF1), Adv (Hexon and Penton), and CMV (pp65 and IE1) antigens, and expansion with IL4+7 in G-Rex devices. The rCTL lines were polyclonal, comprising both CD4+ (33±3%) and CD8+ (60.5±3%) cells, that expressed activation and memory markers. Twenty lines generated from donors that were seropositive for all viruses demonstrated activity against all 3 targets - CMV (IE1: 359±100; pp65: 637±177 SFC/$2x10^5$), EBV (LMP2: 217±60, EBNA1: 67±19 and BZLF1: 111±31) and Adv (Hexon: 265±74, Penton: 191±53) - while 9 lines generated from CMV seronegative donors demonstrated activity exclusively against EBV (LMP2: 197±70, EBNA1: 145±51 and BZLF1: 239±84) and Adv (Hexon: 271±96, Penton: 254±90). None of the T-cell lines reacted against unmodified recipient cells. To date we have administered these lines to 10 allogeneic HSCT recipients at doses ranging from $0.5\text{-}2x10^7/m^2$ as treatment for CMV (n=3), Adv (n=2), EBV (n=2), EBV+Adv (n=1), and CMV+Adv (n=2). One patient developed a skin rash 2 weeks post-rCTLs but no other toxicity have been observed. Eight treated patients, including one with a biopsy-proven EBV lymphoma and the 3 patients with double reactivations, had complete clinical responses to rCTL, which corresponded with an increase in the frequency of virus-specific T-cells detected in peripheral blood. For CMV we saw an increase from a median of 0.5 to 96 and 1 to 277 SFC/$4x10^5$ IE1 and pp65-specific T cells, respectively 3-6wks post-infusion; for Adv an increase from mean 0.5 to 137 and 0.5 to 99 SFC/$4x10^5$ Hexon and Penton-specific cells, respectively, and for EBV an increase from 2.8 to 227, 1.5 to 39, and 1 to 188.5 SFC/$4x10^5$ EBNA1, LMP2, and BZLF1-specific T-cells, respectively. Two patients failed to respond to rCTLs; one with a 3 year history of persistent CMV colitis and one with elevated EBV DNA; both had high pre-existing virus-specific T-cell precursors. rCTLs have been safe and effective in 80% of treated patients and have the potential to increase the availability of cell products for HSCT recipients. We are currently extending this platform to additional viruses.

6

Competitive TNF Inhibitor (ETANERCEPT) for the Treatment of Idiopathic Pneumonia Syndrome (IPS) Following Allogeneic Stem Cell Transplantation (SCT). A Joint Pediatric Blood and Marrow Transplant Consortium (PBMTC) and Childrens Oncology Group (COG) Study

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Idiopathic pneumonia syndrome (IPS) is a noninfectious pulmonary disorder occurring acutely post-SCT, associated